AMENDMENTS TO THE CLAIMS

Claim 1 (Currently amended): A method for assaying homocysteine (Hcy), S-adenosylhomocysteine (SAH) or adenosine in a sample, which method comprises:

a) contacting a sample containing or suspected of containing Hcy, SAH or adenosine with a mutant SAH hydrolase derived from a mammalian SAH hydrolase, wherein said mutant SAH hydrolase has binding affinity for Hcy, SAH or adenosine but has attenuated catalytic activity, and wherein said binding affinity and/or said attenuated catalytic activity of said mutant SAH hydrolase is caused by a mutation in said mutant SAH hydrolase's catalytic site, its binding site for NAD^+, NADH, Hcy, SAH, adenosine, or a combination thereof; and wherein the mutant SAH hydrolase: i) has a mutation in an amino acid residue that participates in catalysis or that is directly interacting with NAD^+, NADH, Hcy, SAH or adenosine; or ii) has a mutation in an amino acid residue that is adjacent to an amino acid residue that participates in catalysis or that is directly interacting with NAD^+, NADH, Hcy, SAH or adenosine; and

b) detecting binding between Hcy, SAH or adenosine with said mutant SAH hydrolase,

whereby the presence or amount of Hcy, SAH or adenosine in said sample is assessed.

Claim 2 (Cancelled):

Claim 3 (Original): The method of claim 1, wherein the mutant SAH hydrolase has enhanced binding affinity for Hcy, SAH or adenosine than a wild type SAH hydrolase from which said mutant SAH hydrolase is derived.

Claim 4 (Original): The method of claim 3, wherein the mutant SAH hydrolase has at least 50 fold higher binding affinity for Hcy, SAH or adenosine than a wild type SAH hydrolase from which said mutant SAH hydrolase is derived.

Claim 5 (Cancelled)

Claim 6 (Original): The method of claim 1, wherein the mutant SAH hydrolase is derived from a human SAH hydrolase.

Claim 7 (Previously presented): The method of claim 1, wherein the mutant SAH hydrolase comprises the amino acid sequence set forth in SEQ ID NO:1 and comprises a mutation selected from the group consisting of R38E, C53S, L54G, T57G, T57S, E59D, N80G, S83G, Y100T, K121A, D131E, D134E, E155G, T157G, T158Y, T159Y, N181A, N191A, L214A, Y221S, K226A, F235S, I240L, N248A, D263G, G269D, R285D, D292G, H301T, K309R, K322G, R329A, L347F, L347Y, L347I, M351A, H353R, S361G, F362S, Y379S, L386A, K388G, H398A, K401R, K401D, T407S, L409G, S420T, P424A, F425S, P427A, D428G, H429A, Y430T, R431K, R431G, Y432S, Y432A, Y432F, and a combination thereof.

Claim 8 (Previously presented): The method of claim 1, wherein prior to the contact between the sample and the mutant SAH hydrolase, oxidized or conjugated Hcy in the sample is converted into reduced Hcy by a reducing agent.

Claim 9 (Original): The method of claim 1, wherein prior to the contact between the sample and the mutant SAH hydrolase, the Hcy in the sample is converted into SAH.

Claim 10 (Previously presented): The method of claim 9, wherein the Hcy in the sample is converted into SAH by a wild-type SAH hydrolase and excess adenosine.

Claim 11 (Previously presented): The method of claim 10, wherein the excess adenosine in the sample is removed by adenosine deaminase while the wild-type SAH hydrolase is inhibited.

Claim 12 (Previously presented): The method of claim 11, wherein the wild-type SAH hydrolase is inhibited by neplanocin A or aristomycin.

Claim 13 (Original): The method of claim 8, further comprising a step of removing the reducing agent used to convert oxidized or conjugated Hcy into reduced Hcy prior to or concurrently with contacting the sample with the mutant SAH hydrolase.

Claim 14 (Original): The method of claim 13, wherein the reducing agent is removed by chromatography.

Claim 15 (Original): The method of claim 14, wherein the chromatography is a batch chromatography.

Claim 16 (Original): The method of claim 1, wherein an indicator dye is used and the indicator dye is removed by chromatography prior to or concurrently with contacting the sample with the mutant SAH hydrolase.

Claim 17 (Original): The method of claim 16, wherein the chromatography is a batch chromatography.

Claim 18 (Currently amended): The method of claim 1, wherein the [[SAH]] sample is contacted with the mutant SAH hydrolase in the presence of a labeled SAH, a labeled SAH derivative, or a labeled SAH analogue, thereby the amount of the labeled SAH, SAH derivative, or SAH analogue bound to the mutant SAH hydrolase inversely relates to the amount of SAH in the sample.

Claim 19 (Previously presented): The method of claim 18, wherein the labeled SAH, derivative, or SAH analogue is labeled with a fluorophore, an enzyme, or a protein.

Claim 20 (Previously presented): The method of claim 19, wherein the labeled SAH, SAH derivative, or SAH analogue is labeled with a fluorecin or a Rocamin, said fluorecin or Rocamin being linked to said SAH, SAH derivative or SAH analogue by a linker of 1-15 carbon atoms in length.

Claim 21 (Previously presented): The method of claim 19, where the enzyme labeled SAH, SAH derivative, or SAH analogue is labeled with a glucose-6-phosphate dehydrogenase (G 6 PDH), an alkaline phosphatase, or a malate dehydrolase, said G-6-PDH, alkaline phosphatase or malate dehydrolase being linked to said SAH, SAH derivative or SAH analogue by a linker of 1-15 carbon atoms in length.

Claim 22 (Previously presented): The method of claim 19, wherein the protein labeled SAH, SAH derivative, or SAH analogue is labeled with a bovine albumin, said bovine albumin being linked to said SAH, SAH derivative or SAH analogue by a linker of 1-15 carbon atoms in length.

Claim 23 (Original): The method of claim 1, wherein the mutant SAH hydrolase is a labeled mutant SAH hydrolase.

Claim 24 (Original): The method of claim 23, wherein the labeled mutant SAH is a fluorescently, enzymatically, biotin or streptavidin labeled mutant SAH hydrolase.

Claim 25 (Original): The method of claim 24, wherein the biotin labeled mutant SAH hydrolase is detected by a streptavidin labeled enzyme.

Claim 26 (Previously presented): The method of claim 25, wherein the streptavidin labeled enzyme is a streptavidin labeled horseradish phosphatase (HRP).

Claim 27 (Original): The method of claim 22, wherein the bovine albumin-SAH conjugate is immobilized.

Claim 28 (Previously presented): The method of claim 19, wherein the fluorophore labeled SAH, SAH derivative, or SAH analogue is directly contacted by the mutant SAH hydrolase, and the resulting change of fluorescent polarization is measured for assessing the presence or amount of Hcy, SAH or adenosine in the sample.

Claim 29 (Previously presented): The method of claim 19, wherein the enzyme labeled SAH, SAH derivative, or SAH analogue is directly contacted by the mutant SAH hydrolase, and the resulting change of enzyme activity is measured for assessing the presence or amount of Hcy, SAH or adenosine in the sample.

Claim 30 (Original): The method of claim 1, wherein the mutant SAH hydrolase is immobilized.

Claim 31 (Original): The method of claim 1, wherein the sample is a body fluid or a biological tissue.

Claim 32 (Original): The method of claim 31, wherein the body fluid is selected from the group consisting of urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus and amniotic fluid.

Claim 33 (Original): The method of claim 31, wherein the body fluid is blood.

Claim 34 (Original): The method of claim 33, wherein the blood sample is further separated into a plasma or serum fraction.

Claim 35 (Original): The method of claim 1, further comprising detecting cholesterol and/or folic acid in the sample.

Claim 36 (Withdrawn): A combination, which combination comprises:

a) a mutant SAH hydrolase that has binding affinity for Hcy, SAH or adenosine but has attenuated catalytic activity, wherein said binding affinity and/or said attenuated catalytic activity of said SAH hydrolase is caused by a mutation in said mutant SAH hydrolase's catalytic site, its binding site for NAD^+, NADH, Hcy, SAH or adenosine, or a combination thereof; and

b) reagents for detecting binding between Hcy, SAH or adenosine and said SAH hydrolase.

Claim 37 (Withdrawn): The combination of claim 36, further comprising a reagent for detecting cholesterol and/or folic acid.

Claim 38 (Withdrawn): A kit, which kit comprises the combination of claim 36.

Claim 39 (Withdrawn): The kit of claim 38, further comprising instructions for assaying Hcy, SAH or adenosine in a sample.

Claim 40 (Withdrawn): An article of manufacture, which article of manufacture comprises:

a) packaging material;

b) a mutant SAH hydrolase that has binding affinity for Hcy, SAH or adenosine but has attenuated catalytic activity, wherein said binding affinity and/or said attenuated catalytic activity of said SAH hydrolase is caused by a mutation in said mutant SAH hydrolase's catalytic site, its binding site for NAD^+, NADH, Hcy, SAH or adenosine, or a combination thereof; and

c) a label indicating that the mutant SAH hydrolase and the means for use in assaying Hcy in a sample.

Claim 41 (Withdrawn): An isolated nucleic acid fragment, which isolated nucleic acid fragment comprises a sequence of nucleotides encoding a mutant SAH hydrolase, wherein said mutant SAH hydrolase comprises the amino acid sequence set forth in SEQ ID NO:1 and comprises a mutation selected from the group consisting of R38E, C53S, L54G, T57G, T57S, E59D, N80G, S83G, Y100T, K121A, D131E, D134E, E155G, T157G, T158Y, T159Y, N181D, N181A, D190A, N191A, L214A, Y221S, K226A, F235S, I240L, N248A, D263G, G269D, R285D, D292G, H301T, K309R, K322G, R329A, L347F, L347Y, L347I, M351A, H353R, S361G, F362S, Y379S, L386A, K388G, H398A, K401R, K401D, T407S, L409G, S420T, P424A, F425S, P427A, D428G, H429A, Y430T, R431K, R431G, Y432S, Y432A, Y432F, and a combination thereof.

Claim 42 (Withdrawn): The isolated nucleic acid fragment of claim 41, wherein the nucleic acid is DNA.

Claim 43 (Withdrawn): The isolated nucleic acid fragment of claim 41, wherein the nucleic acid is RNA.

Claim 44 (Withdrawn): A plasmid, which plasmid comprises the nucleic acid fragment of claim 41.

Claim 45 (Withdrawn): A cell, which cell comprises the plasmid of claim 44.

Claim 46 (Withdrawn): The cell of claim 45 selected from the group consisting of a bacterial cell, a yeast cell, a fungal cell, a plant cell, an insect cell and an animal cell.

Claim 47 (Withdrawn): A method for producing a mutant SAH hydrolase, which method comprises growing the cell of claim 45 under conditions whereby the mutant SAH hydrolase is expressed by the cell, and recovering the expressed mutant SAH hydrolase.

Claim 48 (Withdrawn): A substantially purified mutant SAH hydrolase, wherein said mutant SAH hydrolase comprises the amino acid sequence set forth in SEQ ID NO:1 and comprises a mutation selected from the group consisting of R38E, C53S, L54G, T57G, T57S, E59D, N80G, S83G, Y100T, K121A, D131E, D134E, E155G, T157G, T158Y, T159Y, N181D, N181A, D190A, N191A, L214A, Y221S, K226A, F235S, I240L, N248A, D263G, G269D, R285D, D292G, H301T, K309R, K322G, R329A, L347F, L347Y, L347I, M351A, H353R, S361G, F362S, Y379S, L386A, K388G, H398A, K401R, K401D, T407S, L409G, S420T, P424A, F425S, P427A, D428G, H429A, Y430T, R431K, R431G, Y432S, Y432A, Y432F, and a combination thereof.

Claim 49 (Withdrawn): A conjugate, which conjugate comprises:

a) a mutant SAH hydrolase that has binding affinity for Hcy, SAH or adenosine but has attenuated catalytic activity, wherein said binding affinity and/or said attenuated catalytic activity of said SAH hydrolase is caused by a mutation in said mutant SAH hydrolase's catalytic site, its binding site for NAD^+, NADH, Hcy, SAH or adenosine, or a combination thereof; and

b) a facilitating agent linked to the mutant SAH hydrolase directly or via a linker, wherein the agent facilitates:

i) affinity isolation or purification of a conjugate;

ii) attachment of a conjugate to a surface; or

iii) detection of a conjugate.

Claim 50 (Withdrawn): The conjugate of claim 49, which is a fusion protein.